

A Chromatographic Study of the Influence of Ion Concentrations and pH on the Yield of Volatile Materials from Heat-Treated Natural Product Extracts

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Abstract

The effect of inorganic ion concentrations and pH on the yields of volatile materials from aqueous pressure-cooked solutions of green coffee beans, green tea leaves, and green peanuts as measured by purge and trap-gas chromatography (P&T-GC) with mass spectrometric (MS) detection and flame-ionization detection (FID) is described. Through the use of an internal standard, quantitative descriptions of the effects of these two parameters on the volatile compounds associated with pressure-cooked aqueous extracts of selected natural products are presented. The magnitude of the effect of ion concentration on the amount of material in the headspace is a function of the inorganic compound employed. The higher molecular weight components in the headspace are found to be the most affected by pH and salt concentration. As the concentration of certain inorganic species increases, more of the higher molecular weight components relative to the internal standard are found in the headspace above the pressure-cooked aqueous extracts. Variations in the pH produce similar changes. However, the effect is not universal for the extracts as a group. Increasing the pH results in both increases and decreases in the total amount of volatile material as a function of the aqueous extract type. The ability to exercise control over the release of volatile material from these aqueous extracts by varying the ion concentration and pH of the solutions would seem to provide flexibility in the design of aroma formulations.

Introduction

The perceived aroma and flavor of a wide range of products, such as foods, perfumes, health care products, beverages, and medicines, are a function of the types and levels of volatile organic compounds present in the formulations (1-6). Gas chromatography (GC) has been successfully applied to the separation and qualitative analysis of the components of these mixtures, in part because of the volatile nature of the compounds present in the formulations. A significant number of the analytical applications have employed hyphenated tech-

niques wherein GC is combined with information-rich detectors such as mass spectrometers (MS), infrared (IR) spectrometers, and universal detectors such as flame-ionization detectors (FID). Dynamic technologies, in other words, purge and trap (P&T) and static headspace (E-HS), have been used successfully as methods of sample concentration and introduction of volatile materials into GCs that are coupled to these detectors (6-11).

For a significant number of cases in the literature, reports on the characteristics of the volatile materials from matrices of various types have presented qualitative and component percent distribution analyses of the volatile components. In particular, studies from this laboratory have provided such descriptions of the universal nature of the volatile components from pressure-cooked aqueous extracts of natural products (9,12). These initial reports were followed by an internal standard based quantitative analysis of the volatile components from these extracts (10).

In pure aqueous solutions, volatile compounds are partitioned between the liquid and the gas phases according to their relative volatilities in that particular solution. Among the factors that affect the observed volatility are temperature and concentration. In the case of multicomponent solutions such as coffee and tea, the presence of other nonvolatile dissolved solids will have a significant effect on the distribution of volatile components in the sample headspace (13). For example, the presence of magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) has been shown to affect the perceived aroma and flavor of coffee. In a similar fashion, the addition of salts has been shown to significantly alter the amount of volatile materials observed in the analyses of sweet corn and wine (14,15). In the case of selected forms of cooked sweet corn, both NaCl and sodium carbonate (Na_2CO_3) were added to facilitate the isolation of the volatile components via dynamic headspace procedures. This addition of soluble inorganic species to aqueous solutions would appear to be another application of the "salting out" effect wherein the solubilities of neutral molecules are altered because the polar solvent molecules (e.g., water) are more closely associated with the dissolved cationic and anionic species than

they are with the neutral solutes. It should be mentioned that the addition of Na_2CO_3 to the sweet corn extracts adjusted the pH to between 9.4 and 9.8 and increased the ion concentration to facilitate the isolation of the volatile basic components of the mixtures (15). In a study of the aroma from foods, similar approaches were employed with the addition of NaCl to aqueous solutions of diacetyl. The addition of the salt resulted in a significant increase in the observed volatility of the diacetyl (16). In a study of the volatile components of tomatoes, the use of saturated CaCl_2 aqueous solutions resulted in an increase in the air/water partition coefficient of the volatile compounds (17).

The purpose of this study was to document the effect of ion concentration and pH on the yields of volatile materials from pressure-cooked aqueous extracts of natural products as measured by dynamic headspace techniques. This work describes the systematic examination of the effect of these two parameters on the yields of volatile components from the heat treated aqueous extracts of green coffee beans, green tea leaves, and green peanuts.

Experimental

Extract preparation

The pressure-cooked aqueous extracts were prepared and stored as previously described (9,12). The aqueous extracts were prepared by gently stirring a known amount of the natural product in warm water for about 4 h. After filtration, the solutions were heated in a stirred, sealed reaction vessel at 180°C for 30 min. Upon completion of the heating, the pressure-cooked extracts were rapidly cooled and their headspace profiles determined immediately or they were stored at -20°C until ready for dynamic headspace examination.

Sample preparation

A 21.2 mg/L aqueous cyclohexanone internal standard solution (1 mL) (Aldrich Chemical Company; Milwaukee, WI) was added to 1.0 mL of the pressure-cooked aqueous extract in a 5-mL Tekmar fritless sparge tube (Cincinnati, OH). To six of these resulting solutions, the water-soluble inorganic compound, for example, NaCl , was added in the following amounts: 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 g. The inorganic compound was dissolved by gentle swirling. The resulting samples were affixed to a Tekmar Model 2016 Dynamic Headspace Analysis Unit equipped with individual sample heating units.

The pH of the pressure-cooked aqueous extracts was adjusted by dissolving milligram quantities of K_2CO_3 in the pressure-cooked solutions containing the internal standard until the desired pH range (between 4 and 8) had been reached.

Sample analysis by P&T-GC-MS-FID

The P&T-GC-MS-FID analyses were performed as previously described (9,12). Briefly, after a preheating step of 5 min at 75°C , the aqueous pressure-cooked solutions were sparged with He at 40 mL/min for 20 min, and the contents were deposited on a Tenax trap. The adsorbed materials were thermally transferred from the Tenax trap via heated aluminum-clad deactivated fused-silica tubing to a Hewlett-Packard 5880 GC (Wilmington, DE) set at 5°C followed by temperature programming and analysis by MS and FID. Standard deviations of between 5 and 10% have been documented in the FID area counts measured by this procedure. That trend was found in this work as well.

Results and Discussion

In recent reports on the dynamic headspace analysis of volatiles from selected cooked sweet corn products and tomatoes,

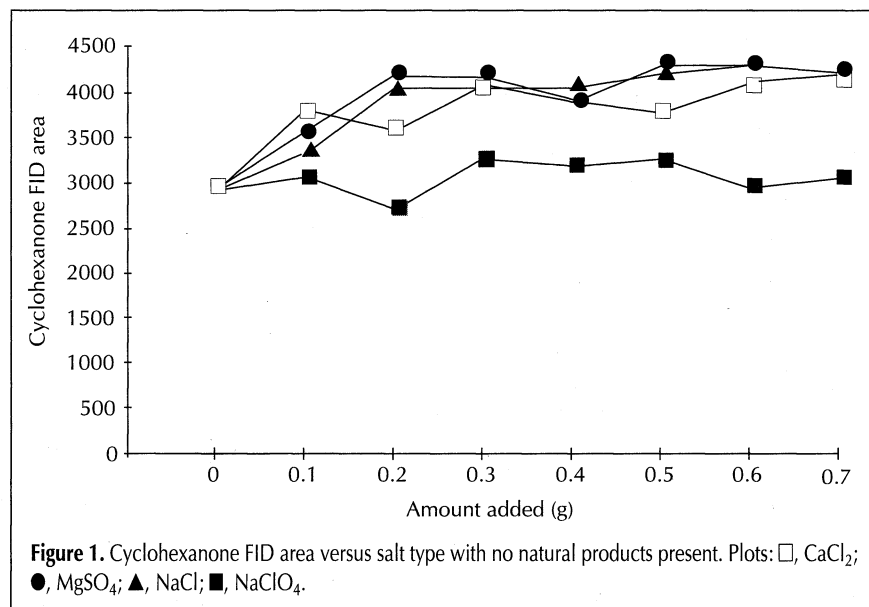


Figure 1. Cyclohexanone FID area versus salt type with no natural products present. Plots: □, CaCl_2 ; ●, MgSO_4 ; ▲, NaCl ; ■, NaClO_4 .

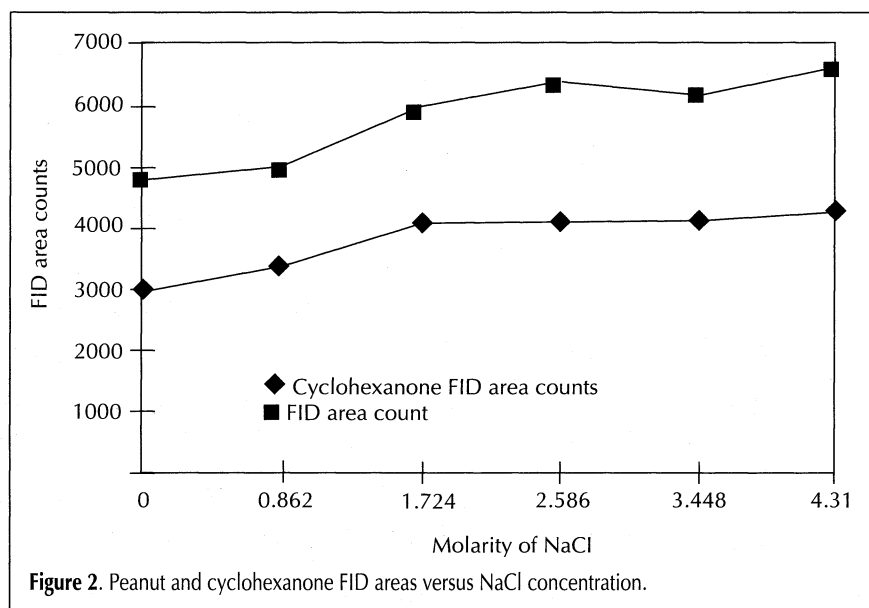


Figure 2. Peanut and cyclohexanone FID areas versus NaCl concentration.

Buttery and co-workers (14,17) employed NaCl, CaCl₂, and NaCO₃ to facilitate isolation of some basic compounds from heat-treated aqueous solutions as the headspace above the cooked samples was swept by purified air. The basic compounds found in the headspace above the cooked sweet corn samples included pyridine and substituted pyrazines. The pH of the cooked sweet corn solutions were adjusted to between 9.4 and 9.8 by the addition of sodium carbonate. Ferreira and co-workers (15) recently noted a similar effect in a Freon 113 extraction of volatile compounds from wine when NaCl, MgSO₄, Na₃PO₄, and (NH₄)₂SO₄ were employed. Esters, alcohols, and acids were shown to be affected by the presence of these salts. Because some of these same organic compounds were found in the headspace above a series of pressure-cooked extracts (9,12), it was of interest to document the effect of the same inorganic compounds and pH on the dynamic headspace profiles obtained in this work.

Prior to investigating the magnitude of the "salting out" effect on the headspace profiles of pressure-cooked aqueous extracts of green coffee beans, green tea leaves, and green peanuts, initial experiments were conducted on deionized water solutions of cyclohexanone, the internal standard employed in previous studies with these extracts (10). Thus, known amounts of

various inorganic compounds were added to deionized water solutions of cyclohexanone, and the resulting cyclohexanone FID area counts were measured (Figure 1) using the P&T-GC-MS-FID approach. The consequences of adding the inorganic compounds were significant in terms of FID area counts for the internal standard, with the exception of NaClO₄. For example, the FID area counts increased from a low of approximately 2800 with no added NaCl to a high of approximately 4200 (an increase of 50%). In contrast, no statistically significant increase in cyclohexanone FID area count was observed with NaClO₄. It was interesting to note that seemingly insignificant increases in cyclohexanone FID area counts were observed after 0.2 g NaCl had been added. This observation was generally true with the two additional salts, MgSO₄ and CaCl₂. This response proved to be one of the essential factors in documenting, quantitatively, the behavior of certain volatile components in the pressure-cooked extracts.

After its effect on the internal standard was documented, identical amounts of NaCl were added to pressure-cooked aqueous solutions of green peanuts, and the total FID area counts for the volatiles were measured. The increase in the total FID area counts from the peanuts as the NaCl concentration increased was somewhat similar to that observed for the standard (Figure 2). However, the trend in the total FID area counts for the pressure-cooked aqueous green peanut extracts appeared to be a slight increase as the NaCl concentration reached saturation at approximately 5.2M. The effect on the FID area counts of the internal standard present in the green peanut heat-treated extract was similar to the effect found when the internal standard was added to distilled water; that is, no significant increase was observed in the internal standard FID area counts after the NaCl concentration reached 1.724M (0.2 g added NaCl). The trend of slightly higher FID area counts as the NaCl concentration increased toward saturation was also evident for the heat-treated aqueous extracts from green tea leaves and green coffee beans (Figure 3). For example, with no added salt the FID area counts from the green tea leaves were 3221.64, whereas the FID area counts for the sample with the highest NaCl concentration were 5313.09 (Table I, Figure 3).

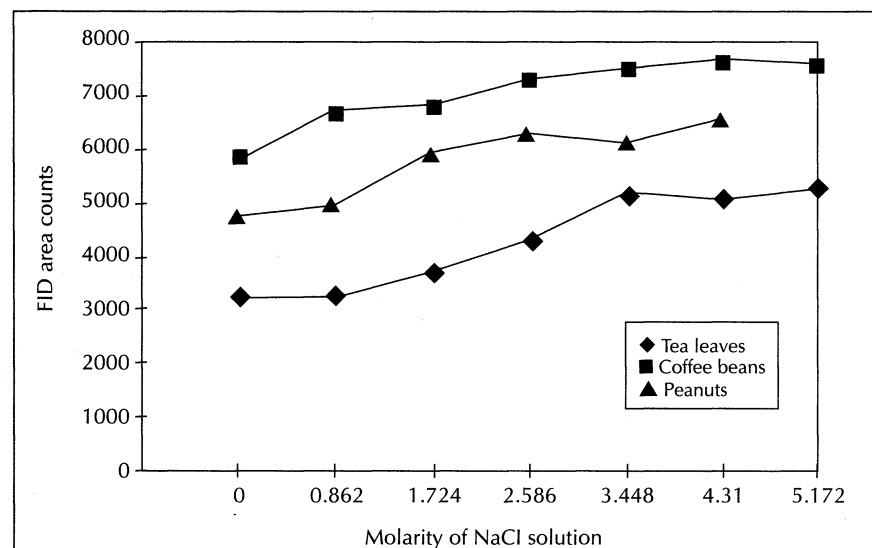


Figure 3. FID area counts for selected heat-treated extracts.

Table I. FID Area Counts for Heat-Treated Aqueous Extracts

Molarity NaCl	Tea leaves	Coffee beans	Peanuts
0	3221.64	5831.37	4793.76
0.862	3229.44	6716.65	5010.37
1.724	3706.13	6802.56	5975.02
2.586	4326.63	7321.52	6346.91
3.448	5185.00	7500.40	6139.29
4.310	5063.19	7663.28	6600.24
5.172	5313.09	7573.53	-

It was of interest to examine the behavior of selected classes of volatile compounds to ascertain if all compounds were being affected equally by the addition of NaCl. For low molecular weight compounds, such as acetone and 3-methylbutanal, in the green coffee bean extract, no significant increases were observed in FID area counts as the NaCl concentration increased (Figure 4A). In contrast, for a higher molecular weight basic compound such as dimethylpyrazine a trend of slightly increasing counts across the entire NaCl concentration range was found. The consequences of added NaCl on the yield of selected compounds such as methylpyrazine, ethyldimethylpyrazine, furfural, and 5-methylfurfural from both peanut and coffee

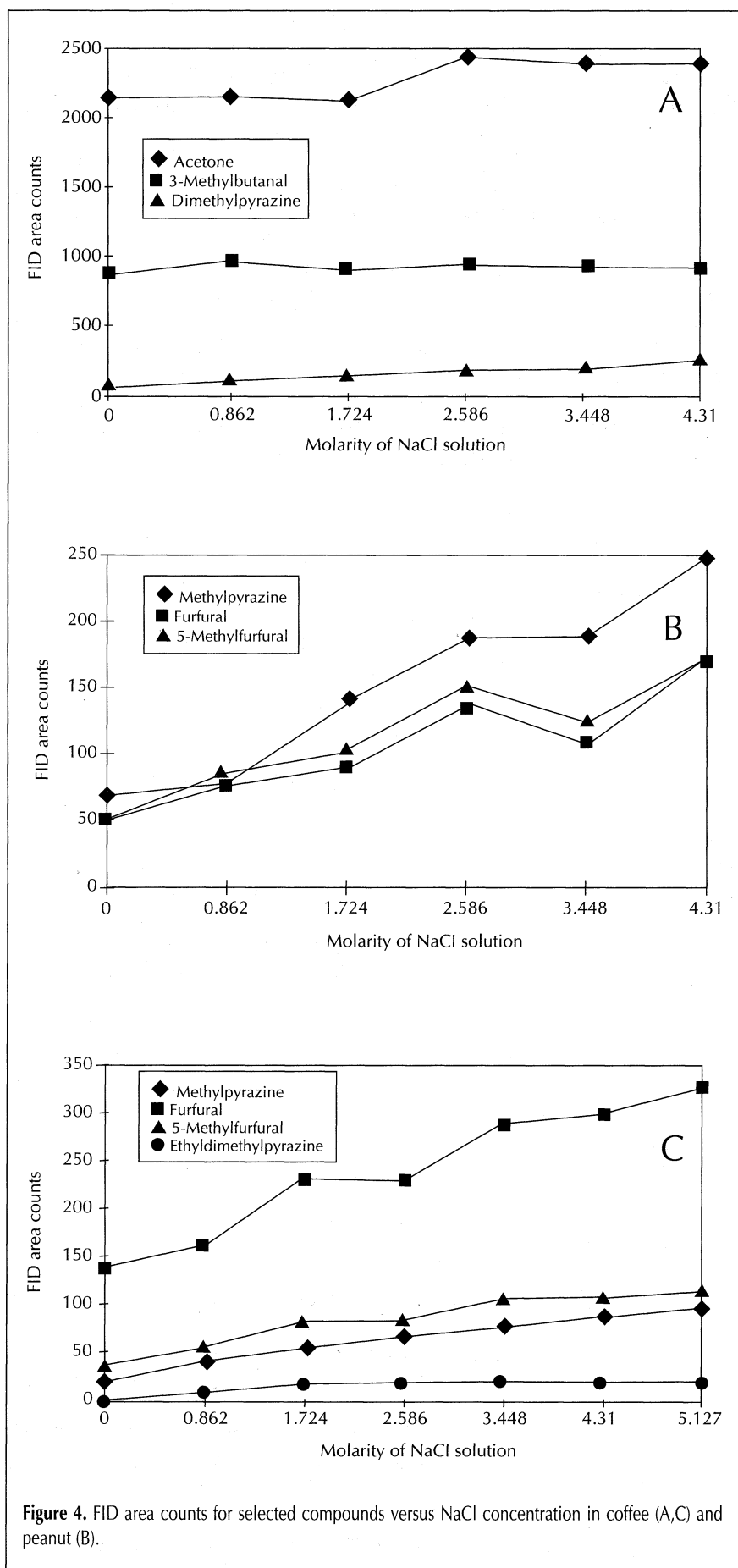


Figure 4. FID area counts for selected compounds versus NaCl concentration in coffee (A,C) and peanut (B).

were significant and are illustrated in Figures 4B and 4C, respectively. For example, the increase in the yield of 5-methylfurfural from the peanut extract from approximately 60 to 230 FID area counts was due to the presence of NaCl.

One of the benefits of employing an internal standard such as cyclohexanone at a known concentration is that concentrations relative to the internal standard for the total and individual volatile compounds in the headspace above the samples can be calculated. For example, the total FID area counts of the volatile material from the green tea leaves, green coffee beans, and green peanuts with no added NaCl were found to be equivalent to 20.75, 39.96, and 34.84 $\mu\text{g/mL}$, respectively. If an internal standard is well behaved and mimics the characteristics of the volatile material in the sample matrix, then it is reasonable to assume that the calculated concentration of volatile material should remain constant, regardless of the amount of NaCl in the matrix. The information shown in Table II revealed this to be generally the case for calculations based on the total FID area counts. The exception to this generalization appeared to be the green tea sample. For example, as the NaCl concentration increased so did the amount of volatile material (from 20.75 to 26.04 $\mu\text{g/mL}$). It appeared that the underlying cause for this behavior might rest with discrepancies between the behaviors of the internal standard and the higher molecular weight compounds at a specific NaCl concentration. The behavior of two compounds, furfural and 5-methylfurfural (Table III) relative to the internal standard, provided the rationale for this hypothesis. It was obvious that significant increases in the yield of these two compounds occurred through out the entire range of NaCl concentrations while less significant increases were observed for the internal standard. It was not surprising then to find no such significant increase for benzaldehyde or ethyldimethylpyrazine across the entire range of NaCl concentrations examined in the green tea sample. Thus, the observed behavior can most likely be attributed to significant differences in the influence of NaCl on the volatility behaviors of selected compounds relative to that of the internal standard, cyclohexanone. It could be reasoned that similar behaviors could be occurring with the other two extracts but that the magnitude of the effect must be much less severe in the cases of green coffee bean and green peanut extracts.

To examine the scope of the effect of ion concentration and type, additional studies similar to those conducted with NaCl were completed on the heat-treated aqueous peanut extracts. CaCl_2 , MgSO_4 , and NaClO_4 were used. Earlier indications suggested that, based on the performance of the internal standard (cyclohexanone), NaClO_4 could have a unique effect on the volatility of the components in aqueous extracts when compared with the other salts. Figure 5 provides evidence that this

was the case for the peanut extract. The other extracts demonstrated similar behavior when NaClO_4 was added. No substantial increase in the FID area count was observed for extracts spiked with NaClO_4 throughout the entire NaClO_4 concentration range. These results were in marked contrast to those obtained for the peanut extract spiked with the salts. In other words, the following is true:

- Extracts spiked with NaCl showed a marked increase in the amount of volatile materials whereas the addition of NaClO_4 resulted in no substantial increase in volatile FID area counts.
- The addition of CaCl_2 and MgSO_4 to the extracts resulted in increases in the FID area counts of all of the extracts. These increases were intermediate between those observed with NaCl and NaClO_4 . For example, after addition of 0.6 g NaCl, MgSO_4 , CaCl_2 , and NaClO_4 to the peanut extract, the resultant FID area counts of the spiked peanut extracts were 6700.01, 5467.22, 5444.69, and 3010.89, respectively. This behavior was repeated for the coffee and tea extracts.

Calculations of the concentration of volatile material as a function of the amount and type of salt were made using the response of the internal standard. No statistically significant differences in concentration were found regardless of the type or amount of salt employed. For example, even if the unique behavior of the green tea leaves/NaCl experiment is included in the calculations, the total amount of volatile material in the green tea, green coffee, and green peanut extracts were 21.82 ± 1.00 , 36.97 ± 2.30 , and 29.84 ± 2.74 $\mu\text{g/mL}$, respectively, regardless of the type and amount of salt used.

In summary, results from dynamic headspace techniques indicated that NaCl has a significant effect on the release of volatile components from aqueous extracts, whereas the effects were much less dramatic for other water-soluble inorganic compounds examined in this study. None of the salts, when employing an internal standard, produced statistically significant changes in the calculated concentration of volatile material in the extracts.

Changes in the FID area counts of the pressure-cooked aqueous extracts due to fluctuations in pH were pronounced but not as significant as those changes due to addition of NaCl. For example, the FID area counts for the peanut extract increased only slightly from 4903.77 to 5867.74 when the pH increased from 4.3 to 8.3 (Table IV).

In marked contrast to the effect of varying the concentration of inorganic salt, there was no statistically significant change in the FID area counts of the internal standard (IS) as a function of pH. All cyclohexanone FID values were within experimental error

Molarity NaCl	Tea leaves ($\mu\text{g/mL}$)	Coffee beans ($\mu\text{g/mL}$)	Peanuts ($\mu\text{g/mL}$)
0	20.75	39.96	34.85
0.86	20.75	37.25	31.73
1.72	19.22	37.15	31.12
2.59	23.18	37.35	32.94
3.45	23.62	37.81	31.87
4.31	26.61	38.67	32.94
5.17	26.03	37.11	

Molarity NaCl	Furfural ($\mu\text{g/mL}$)	Benzaldehyde ($\mu\text{g/mL}$)	5-Methylfurfural ($\mu\text{g/mL}$)	Ethylidimethylpyrazine ($\mu\text{g/mL}$)
0	7.94	0.30	1.65	0.69
0.86	7.46	0.31	1.61	0.72
1.72	7.73	0.27	1.64	0.55
2.59	9.81	0.26	2.22	0.70
3.45	9.82	0.30	2.46	0.60
4.31	12.25	0.29	3.15	0.71
5.17	11.59	0.28	2.95	0.69

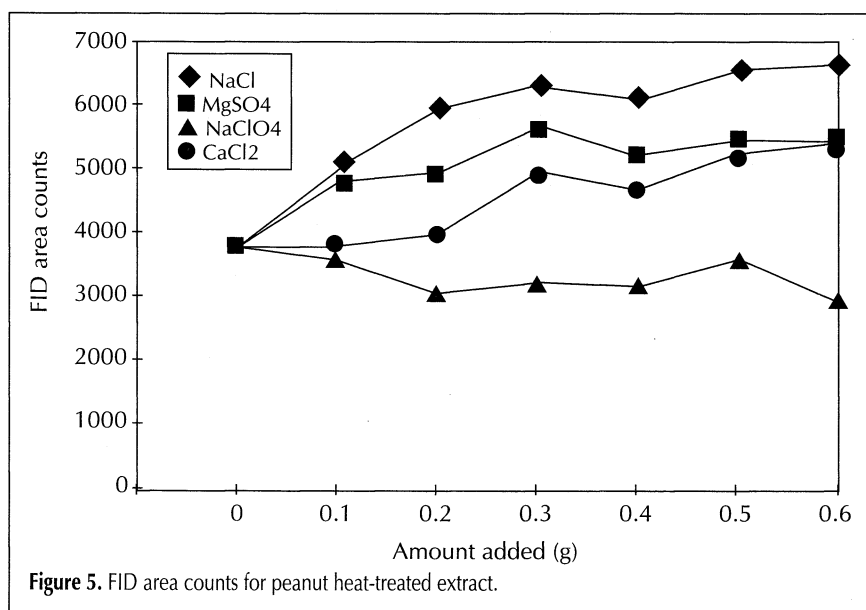


Figure 5. FID area counts for peanut heat-treated extract.

Table IV. Effects of pH on Extract FID Area Counts

Sample	pH	FID area counts			Conc. ($\mu\text{g/mL}$)
		Total	IS	Volatile	
Peanut	4.3	7852.78	2949.01	4903.77	35.25
	6.8	7938.06	2955.32	4982.74	35.74
	8.3	8897.19	3029.45	5867.74	41.06
Coffee	4.7	9655.99	2994.58	6661.41	47.16
	5.9	7740.76	2710.43	5030.33	39.35
	7.6	8106.95	2920.46	5186.49	37.65
Tea	4.4	6328.25	3104.66	3223.59	22.01
	6.4	6602.13	3049.15	3552.98	24.70
	7.7	7071.89	3237.38	3834.51	25.11

regardless of the extract or the pH (Table IV). For example, the average FID area counts for the internal standard were $2994.49 \pm 4.78\%$ throughout all of the pH values and all of the extracts. In contrast, increases in FID area counts on the order of 75% were seen for the internal standard with added NaCl.

Thus, with constant responses observed for the internal standard, any changes in the FID area counts for the volatiles as a function of pH would translate directly into changes in the calculated concentrations (Table IV). Slight increases in the volatile materials were observed for the green peanut and the green tea samples, but the amount of volatile material from the coffee extract showed a slight decreasing trend (Table IV). This extract-dependent behavior was similar to that observed in the green tea leaves/NaCl experiment, wherein the tea extract showed slight increases in the amount of volatile materials with increases in salt (while the other two extracts showed no significant increases). The FID area counts of all of the major components of all of the extracts were consistent with the trends displayed by the total FID area count when the pH was adjusted.

Conclusion

By employing a dynamic headspace-gas chromatography hyphenated technique, it was possible to document the effect of inorganic salt concentration on the yields of volatile materials from pressure-cooked aqueous extracts of natural materials. By combining the performance of an internal standard, cyclohexanone, with a mass spectrometer and a flame-ionization detector, a description of the headspace behavior of total and specific compounds in the presence of known amounts of selected inorganic salts was obtained. These findings document that, as the amount of salt varies, compounds differing in functional groups and molecular weight may display significant differences in the characteristics of their headspace volatility relative to that of an internal standard. Based on the performance of an internal standard, no statistically significant differences in the concentrations of volatile materials were observed to be a function of the type of salt used. Manipulation of the solution pH by

the addition of K_2CO_3 resulted in meaningful changes in the observed concentration of volatile materials.

These results have implications for the aroma and flavor industry in that quantitative assessments can now be made on the effects of pH and dissolved inorganic solids such as NaCl. Such information should yield additional flexibility in the design and formulation of new aromas.

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Manuscript accepted June 29, 1995.